

Oral Gabapentin Activates Spinal Cholinergic Circuits to Reduce Hypersensitivity after Peripheral Nerve Injury and Interacts Synergistically with Oral Donepezil

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Background: Gabapentin administration into the brain of mice reduces nerve injury-induced hypersensitivity and is blocked by intrathecal atropine and enhanced by intrathecal neostigmine. The authors tested the relevance of these findings to oral therapy by examining the efficacy of oral gabapentin to reduce hypersensitivity after nerve injury in rats and its interaction with the clinically used cholinesterase inhibitor, donepezil.

Methods: Male rats with hypersensitivity after spinal nerve ligation received gabapentin orally, intrathecally, and intracerebroventricularly with or without intrathecal atropine, and withdrawal threshold to paw pressure was determined. The effects of oral gabapentin and donepezil alone and in combination on withdrawal threshold were determined in an isobolographic design.

Results: Gabapentin reduced hypersensitivity to paw pressure by all routes of administration, and was more potent and with a quicker onset after intracerebroventricular than intrathecal injection. Intrathecal atropine reversed the effect of intracerebroventricular and oral gabapentin. Oral gabapentin and donepezil interacted in a strongly synergistic manner, with an observed efficacy at one tenth the predicted dose of an additive interaction. The gabapentin–donepezil combination was reversed by intrathecal atropine.

Conclusions: Although gabapentin may relieve neuropathic pain by actions at many sites, these results suggest that its actions in the brain to cause spinal cholinergic activation predominate after oral administration. Side effects, particularly nausea, cannot be accurately determined on rats. Nevertheless, oral donepezil is well tolerated by patients in the treatment of Alzheimer dementia, and the current study provides the rationale for clinical study of combination of gabapentin and donepezil to treat neuropathic pain.

NEUROPATHIC pain represents an unmet medical need, in part due to poor efficacy of existing treatments and in part due to their side effects and expense, which limit their effective application. Gabapentin was licensed as an antiepileptic drug in 1993 and has been approved as a safe and effective treatment of neuropathic pain.¹ Nonetheless, gabapentin is not effective in all patients and is limited in others by side effects, especially on cognition.¹ Gabapentin's efficacy depends on its action

on the $\alpha_2\delta$ subunit of calcium channels,^{2,3} but the circuits activated by gabapentin for analgesia are not entirely known. We and others recently demonstrated that gabapentin activates the descending bulbospinal noradrenergic pathway in mice after nerve injury⁴ and in rats after surgical incision.⁵ Norepinephrine is released in the spinal dorsal horn by descending inhibitory noradrenergic axons, which mainly originate from the locus ceruleus and adjacent nuclei in the brainstem, and suppresses the activation of spinal nociceptive neurons *via* activation of α_2 adrenoceptors.⁶ We also showed clinical relevance of these findings by demonstrating that orally administered gabapentin significantly increases norepinephrine concentration in cerebrospinal fluid and decreases morphine requirements after surgery in patients with chronic pain.⁵ These findings argue for activation of descending bulbospinal noradrenergic pathway as one of the pivotal mechanisms of gabapentin analgesia, at least after surgery. In the current study, we extended these observations by testing the noradrenergic dependency of gabapentin's effect after oral, intrathecal, and intracerebroventricular administration after spinal nerve injury in rats, a model of neuropathic pain.

Spinally released norepinephrine stimulates α_2 adrenoceptors, which in turn activate spinal cholinergic circuits in humans and animals.⁷ In mice with nerve injury, intracerebroventricular gabapentin analgesia is completely blocked by intrathecal atropine and potentiated by intrathecal neostigmine,⁸ consistent with a supraspinal effect of gabapentin to activate this descending noradrenergic–cholinergic cascade. On the other hand, gabapentin also reduces ectopic firing of injured peripheral nerves⁹ and reduces central sensitization in the spinal cord.¹⁰ A second purpose of the current study was to test whether intrathecal atropine, which would block only the descending inhibitory mechanism of gabapentin, alters efficacy of gabapentin when it is given by the clinically relevant oral route of administration.

We recently reported that oral administration of the cholinesterase inhibitor donepezil (Aricept; Pfizer, New York, NY) produced an analgesic effect after nerve injury in rats by spinal muscarinic receptor activation.¹¹ Donepezil is currently approved for the treatment of Alzheimer dementia and is well tolerated in elderly patients.¹² This is in stark contrast to the severe nausea and vomiting produced by intrathecal neostigmine when administered for analgesia.¹³ Our previous study showed that oral donepezil maintained efficacy over 2 weeks of twice-daily administration, and this treatment did not

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lead to desensitization of muscarinic receptor-coupled G proteins in brain or spinal cord.¹¹ The final purpose of the current study was to test the hypothesis that gabapentin and donepezil would potentiate each other when administered by the clinically relevant oral route and to quantify the type of potentiation.

Materials and Methods

Animals

Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) weighing 200–250 g were used in this study. Animal surgery conformed to the Wake Forest University Guidelines on the ethical use of animals, and studies were performed under Animal Care and Use Committee approval from Wake Forest University School of Medicine, Winston-Salem, North Carolina. Animals were housed under a 12-h light-dark cycle, with food and water *ad libitum*.

Surgical Preparations

Spinal Nerve Ligation. As previously described,¹⁴ animals were anesthetized with inhalational halothane in oxygen, the lateral laminae of lower lumbar and upper sacral vertebrae were exposed, the right L6 transverse process was removed, and the right L5 and L6 spinal nerves were identified and tightly ligated using 6-0 silk suture. The wound was closed and animals were allowed to recover for 2 weeks.

Intrathecal Catheterization. Animals were anesthetized with halothane and intrathecal catheters were implanted as previously described.¹⁵ Animals were placed prone in a stereotaxic frame, and a small incision was made at the back of the neck. A small puncture was made in the atlanto-occipital membrane of the cisterna magnum, and a polyethylene catheter, 8.5 cm, was inserted so that the caudal tip reached the lumbar enlargement of the spinal cord. The rostral end of the catheter was exteriorized at the top of the head and the wound was closed with sutures.

Intracerebroventricular Catheterization. Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and atropine (0.1 mg/kg), and intracerebroventricular catheters were implanted as previously described.⁵ Briefly, animals were placed securely in a stereotaxic device (KOPF, Tujunga, CA), and a sterile stainless steel guide cannula (22-gauge needle shaft; Plastics One, Roanoke, VA) was implanted into the left lateral cerebral ventricle. The coordinates for the placement of the tip of the guide cannula were 0.80 mm posterior and 1.5 mm lateral to the bregma, and 3.5 mm ventral from the surface of the dura mater, according to the rat brain atlas.¹⁶

After implantation of the intracerebroventricular and/or intrathecal catheters, rats were housed individu-

ally with free access to food and water. Animals were allowed at least 5 days to recover from the surgery. One rat was excluded from the current study and killed because of motor dysfunction.

Behavioral Test

Withdrawal threshold to pressure applied to the hind paw, expressed in grams, was measured using an analgesimeter (Ugo Basile, Comerio, Italy) as previously described.¹⁷ The device applies increasing pressure to the hind paw. When the animal withdrew the paw or vocalized, the pressure was immediately released, and the nociceptive threshold was read on a scale. A cutoff of 250 g was used to avoid potential tissue injury. All animals were trained for 3–5 days with this measurement before recording baseline values.

Drugs and Administration

All drugs were purchased from Sigma (St. Louis, MO) except gabapentin solution for oral administration (Neurontin® 50 mg/ml solution; Park-Davis, New York, NY) and donepezil (Aricept®, Pfizer, New York, NY). For oral administration, gabapentin was diluted with sterilized water (6 ml/kg), and donepezil pills were crushed with a mortar and dissolved in 0.5% carboxymethylcellulose solution (5 ml/kg), and then given to animals by gastric lavage. For the combination drug study, oral gabapentin and donepezil were administered 120 and 60 min before the measurement, respectively, with fixed ratio dosing (gabapentin:donepezil = 10:1). For intracerebroventricular administration, gabapentin hydrochloride powder was dissolved in saline and injected (3–100 µg/5 µl/rat). For intrathecal administration, gabapentin hydrochloride powder, atropine sulfate, and idazoxan hydrochloride were dissolved in saline and were injected (10 µl/rat) followed by 10 µl saline.

Statistical Analyses

Unless otherwise stated, data are presented as mean ± SEM. Differences among groups for withdrawal threshold were determined using one- or two-way analysis of variance. To calculate the effective dose to produce a 50% maximum effect (ED₅₀) of each drug, the response threshold data were converted to a percentage of return to presurgery threshold according to the following formula: % return to presurgery threshold = (postdrug threshold – baseline predrug threshold)/(pre-SNL threshold – baseline predrug threshold) × 100. Predrug threshold was the withdrawal threshold after spinal nerve ligation (SNL). ED₅₀ was determined using linear regression for each drug. Isobolographic analysis at the ED₅₀ level of effect was performed as described previously.¹⁸ The significance level was set at $P < 0.05$.

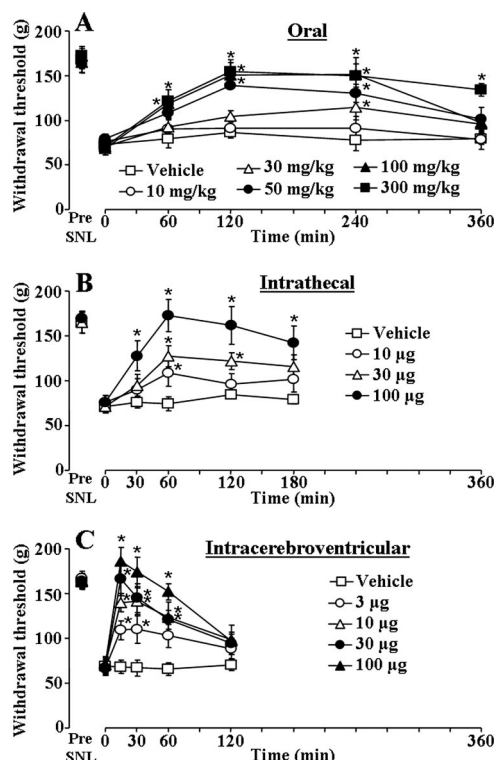


Fig. 1. Effects of orally, intrathecally, and intracerebroventricularly administered gabapentin after spinal nerve ligation (SNL) in rats. The mechanical withdrawal threshold is presented over time. Oral (10–300 mg/kg, $n = 6$; A), intrathecal (10–100 μ g/rat, $n = 6$; B), and intracerebroventricular (3–100 μ g/rat, $n = 6$ –8; C) gabapentin produced a dose-dependent increase in withdrawal threshold compared with vehicle. * $P < 0.05$ versus time 0 by one-way analysis of variance. Groups differ by two-way analysis of variance, with (300 mg/kg, 100 mg) > (30 mg/kg, 10 mg/kg, vehicle), 50 mg/kg > (10 mg/kg, vehicle), 30 mg/kg > vehicle in A; with 100 μ g > (30 μ g, 10 μ g, vehicle), (30 μ g, 10 μ g) > vehicle in B; and with 100 μ g > (3 μ g, vehicle), (30 μ g, 10 μ g, 3 μ g) > vehicle in C.

Results

Spinal nerve ligation strongly decreased the withdrawal threshold of the hind paw ipsilateral to ligation from 163 ± 24 g to 71 ± 16 g (mean \pm SD, $P < 0.0001$, $n = 184$). In the current study, we also observed that the withdrawal threshold in the contralateral hind paw was slightly but significantly decreased from 166 ± 27 g to 134 ± 23 g (mean \pm SD, $P < 0.0001$, $n = 116$), similar to our previous report.¹¹

Dose-Response of Gabapentin

Orally, intrathecally, and intracerebroventricularly administered gabapentin produced analgesia in the hind paw ipsilateral to SNL in a dose-dependent manner (fig. 1). Oral gabapentin showed significant analgesic effects from 30 to 300 mg/kg compared with vehicle ($P < 0.05$; fig. 1A). The peak effect of oral gabapentin was observed 120–240 min after administration. The ED₅₀ value (95% confidence interval) of oral gabapentin calculated at the 120-min time point was 44 mg/kg (23–84 mg/kg). Intra-

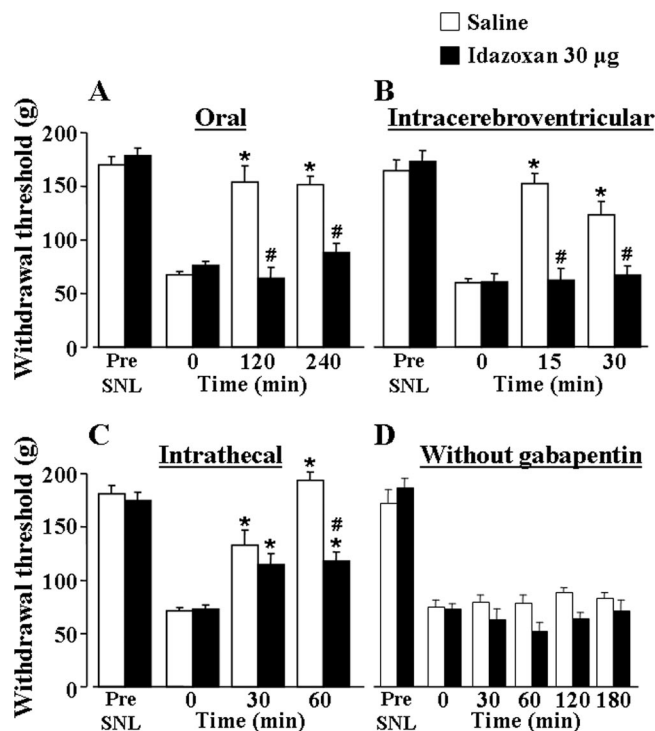


Fig. 2. Effects of intrathecal idazoxan on oral, intrathecal, and intracerebroventricular gabapentin analgesia after spinal nerve ligation (SNL) in rats. The mechanical withdrawal threshold is presented over time. (A) Intrathecal idazoxan or saline was injected 90 min after oral gabapentin (100 mg/kg, $n = 6$ –7). (B and C) Intrathecal idazoxan or saline was coadministered with intracerebroventricular (30 μ g/rat, $n = 6$; B) and intrathecal (100 μ g/rat, $n = 6$; C) gabapentin. (D) Intrathecal idazoxan or saline was injected alone ($n = 6$). * $P < 0.05$ versus time 0 by one-way analysis of variance. Groups differ by two-way analysis of variance in A, B, and C but not in D. # $P < 0.05$ versus saline by two-way analysis of variance followed by Student-Newman-Keuls test.

thecally administered gabapentin produced significant analgesia from 10 to 100 μ g/rat compared with vehicle ($P < 0.05$; fig. 1B). The peak effect of intrathecal gabapentin was observed 60 min after administration. The ED₅₀ value (95% confidence interval) of intrathecal gabapentin calculated at the 60-min time point was 16 μ g/rat (7.3–34 μ g/rat). Intracerebroventricularly administered gabapentin produced significant analgesia from 3 to 100 μ g/rat compared with vehicle ($P < 0.05$; fig. 1C). The peak effect of intracerebroventricular gabapentin was observed 15 min after administration. The ED₅₀ value (95% confidence interval) of intracerebroventricular gabapentin calculated at the 15-min time point was 4.1 μ g/rat (1.9–8.6 μ g/rat).

Effect of Intrathecal α_2 -Adrenoceptor Antagonist on Gabapentin Analgesia

Intrathecal administration of the α_2 -adrenoceptor antagonist idazoxan (30 μ g/rat) completely blocked oral (100 mg/kg) and intracerebroventricular (30 μ g/rat) gabapentin effects (figs. 2A and B), in accord with previous studies in mice after nerve injury⁴ and in rats after

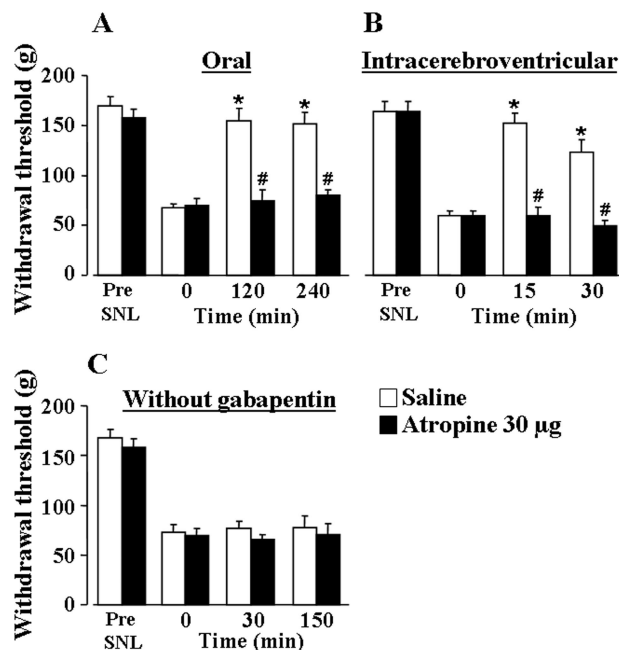


Fig. 3. Effects of intrathecal atropine on oral and intracerebroventricular gabapentin analgesia after spinal nerve ligation (SNL) in rats. The mechanical withdrawal threshold is presented over time. (A) Intrathecal atropine or saline was injected 90 min after oral gabapentin (100 mg/kg, $n = 6-7$). (B) Intrathecal atropine or saline was coadministered with intracerebroventricular gabapentin (30 µg/rat, $n = 6$). (C) Intrathecal atropine or saline was injected alone ($n = 6$). * $P < 0.05$ versus time 0 by one-way analysis of variance. Groups differ by two-way analysis of variance in A and B but not in C. # $P < 0.05$ versus saline by two-way analysis of variance followed by Student-Newman-Keuls test.

incisional surgery.⁵ However, intrathecal idazoxan only blocked the effect of intrathecal gabapentin at later times (60 min) (fig. 2C). Idazoxan alone did not affect withdrawal threshold in the paw ipsilateral to the nerve injury (fig. 2D).

Effect of Muscarinic Antagonist on Gabapentin Analgesia

Because α_2 -adrenoceptor stimulation induces acetylcholine release and produces analgesia mediated by muscarinic receptors⁷ and because muscarinic antagonists blocked the antihypersensitivity effect of intracerebroventricular gabapentin in mice,⁸ we examined the effect of intrathecal injection of the nonselective muscarinic antagonist atropine (30 µg/rat) on oral and intracerebroventricular gabapentin. This dose of atropine was based on previous studies.^{5,19,20} Intrathecal atropine completely blocked the effects of oral (100 mg/kg) and intracerebroventricular (30 µg/rat) gabapentin on paw withdrawal threshold (figs. 3A and B). Intrathecal atropine alone did not affect withdrawal threshold in the paw ipsilateral to SNL (fig. 3C).

Interaction of Donepezil and Gabapentin

Oral administration of the cholinesterase inhibitor donepezil produced a dose-dependent increase in paw

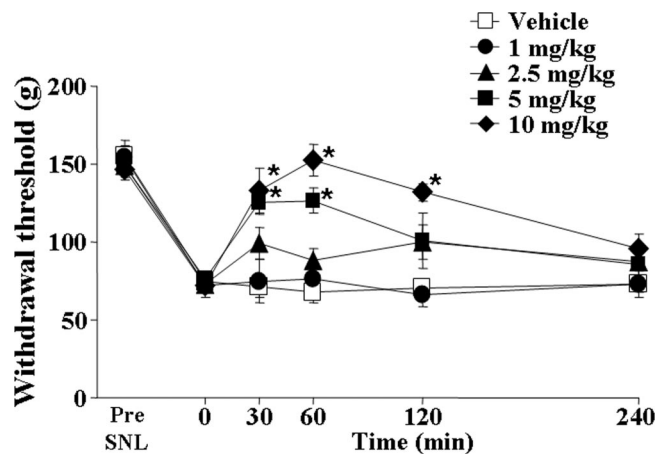


Fig. 4. Effects of orally administered donepezil after spinal nerve ligation (SNL) in rats. The mechanical withdrawal threshold is presented over time. Oral donepezil (1–10 mg/kg, $n = 6-8$) produced a dose-dependent increase in withdrawal threshold compared with vehicle. * $P < 0.05$ versus time 0 by one-way analysis of variance. Groups differ by two-way analysis of variance, with 10 mg > (2.5 mg/kg, 1 mg/kg, vehicle) and 5 mg/kg > (1 mg/kg, vehicle).

withdrawal threshold in doses of 5–10 mg/kg compared with vehicle ($P < 0.05$; fig. 4). The peak effect of oral donepezil was observed 30–60 min after administration, with an ED₅₀ value (95% confidence interval) of 3.1 mg/kg (2.2–4.5 mg/kg). The combination of oral gabapentin with donepezil in a 1:10 ratio produced a dose-dependent analgesia in the paw ipsilateral to SNL (fig. 5A), with an ED₅₀ value (95% confidence interval) of 4.3 mg/kg (3.2–5.7 mg/kg). Rats receiving this combination showed normal grooming and exploration activity. Isobolographic analysis indicated that there was significant difference between the confidence intervals of the experimentally determined combination ED₅₀ and the theoretical ED₅₀ of additivity (40 mg/kg; 21–76 mg/kg), indicating a synergistic interaction ($P < 0.05$; fig. 5B). Intrathecally administered atropine (30 µg/rat) completely blocked the effect of combination of gabapentin (12.5 mg/kg) with donepezil (1.25 mg/kg) (fig. 6).

Discussion

Peripheral nerve injury can result in chronic pain, hyperalgesia, and allodynia, often exhibiting poor response to traditional analgesics. Opioids are sometimes used in this setting, but their chronic administration is fraught with dose escalation and side effects. For these reasons, alternative treatments have been sought for decades. Gabapentin was licensed as an antiepileptic drug in 1993 and has subsequently been approved in the treatment of some forms of neuropathic pain.²¹ Donepezil is a currently approved cholinesterase inhibitor for the treatment of Alzheimer dementia and is well tolerated in elderly patients.¹² The current study extends previous observations to suggest that these oral thera-

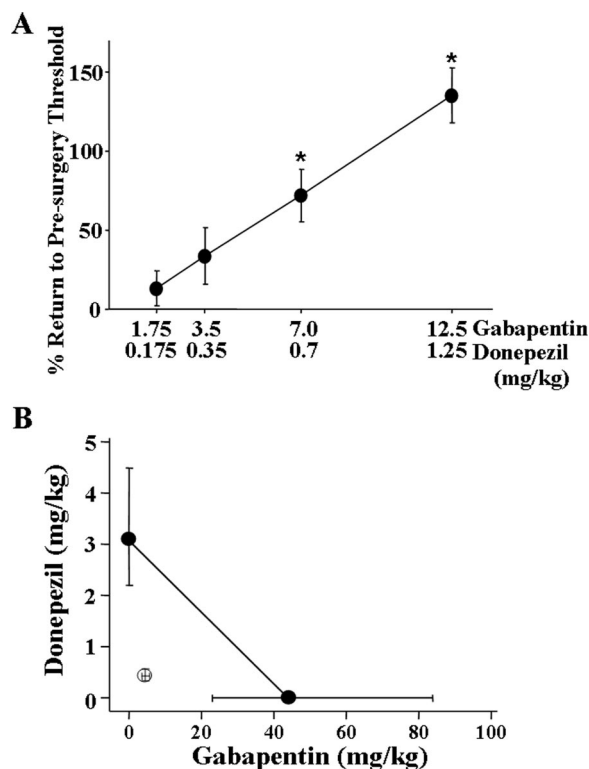


Fig. 5. Interaction between oral gabapentin and donepezil after spinal nerve ligation in rats. (A) Oral gabapentin and donepezil were administered 120 and 60 min before the measurement, respectively, with fixed ratio dosing (gabapentin:donepezil = 10:1, $n = 6-7$). Data represent a percentage of return to presurgery threshold (see Materials and Methods for details). * $P < 0.05$ versus predrug value for each dose by one-way analysis of variance. (B) Closed circles represent ED_{50} values with 95% confidence intervals for each agent and are plotted on the x-axis and y-axis, respectively. The line connecting these two points is the theoretical line of additivity. The open circle represents the experimentally observed ED_{50} point with 95% confidence interval for the drug combination calculated from data of A. The ED_{50} for the drug combination lies below the additive line, indicating synergy ($P < 0.05$ by z test).

pies, neither of which was developed for pain treatment, converge on an analgesic pathway after nerve injury and may be a powerful combination for treatment of neuropathic pain.

Activation of Descending Noradrenergic Pathway by Gabapentin

Gabapentin has a high affinity for the $\alpha_2\delta$ subunit of voltage-gated calcium channels,²² which modulate the release of excitatory amino acids at the level of the spinal dorsal horn and which are present in many other sites in the central nervous system. $\alpha_2\delta$ subunits are up-regulated after nerve injury in animals,² and transgenic mice experiments confirm the importance of these subunits to the antihypersensitivity effects of gabapentin after nerve injury.³ These studies identify a molecular target for gabapentin action, but not the anatomy or circuits affected by this target. Because spinal plasticity and sensitization after nerve injury are recognized to play pivotal

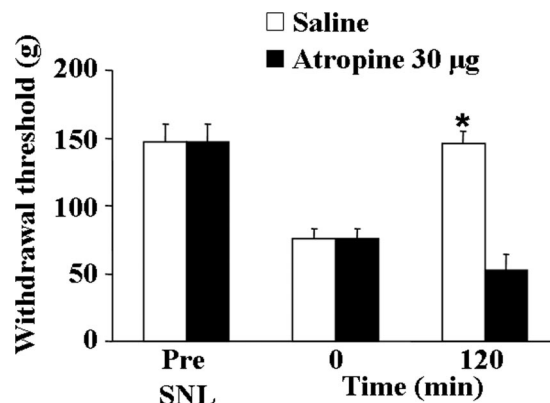


Fig. 6. Effect of intrathecal atropine on oral gabapentin–donepezil combination analgesia after spinal nerve ligation (SNL) in rats. The mechanical withdrawal threshold is presented over time. Oral gabapentin (12.5 mg/kg) and donepezil (1.25 mg/kg) were administered 120 and 60 min before the measurement, respectively. Intrathecal atropine ($n = 8$) or saline ($n = 8$) was injected 30 min before the measurement. * $P < 0.05$ versus time 0 by one-way analysis of variance. Groups differ by two-way analysis of variance.

roles in neuropathic pain, most studies have focused on peripheral afferents and spinal cord neurons as sites of action of gabapentin. Tanabe *et al.*⁴ recently reported, however, that gabapentin also acts supraspinally to stimulate the descending bulbospinal noradrenergic pathway in mice after partial sciatic nerve ligation. We also recently demonstrated that intracerebroventricular and oral gabapentin produced a descending noradrenergic pathway-dependent analgesia in rats 24 h after paw incision and that orally administered gabapentin increased norepinephrine concentration in cerebrospinal fluid and decreased morphine requirements after surgery in patients with chronic pain.⁵ In the current study, we extended these perioperative observations to the chronic nerve injury state.

The greater potency and more rapid onset of gabapentin after intracerebroventricular than intrathecal injection is consistent with a prominent action of gabapentin in the brain. We speculate that the delayed onset of antihypersensitivity for gabapentin after intrathecal injection and the delay in its blockade by intrathecal idazoxan are consistent with slow spread of gabapentin to supraspinal sites of action after intrathecal injection, where it activated the descending noradrenergic pathways. This speculation is supported by recent report that intracerebroventricular but not intrathecal gabapentin stimulates spinal noradrenergic activity early (15 min) after injection in mice after peripheral nerve injury.²³ Peripheral nerve injury increases sensitivity of spinal neurons to α_2 -adrenoceptor agonists such as clonidine²⁴ and increases the descending noradrenergic fiber density in the spinal dorsal horn.²⁵ Because systemic or intracerebroventricular gabapentin did not produce antinociception in normal animals but reduced hypersensitivity in animals with peripheral nerve injury,^{4,9} one

could argue that functional and anatomical plasticity of the descending noradrenergic system after nerve injury plays one of the key roles for enhanced gabapentin analgesia in neuropathic pain.

The mechanisms by which gabapentin activates noradrenergic neurons in the brainstem are unknown. A direct effect seems unlikely, because gabapentin inhibits rather than excites norepinephrine release in other sites in the brain.²⁶ Although further studies are still required to clarify the supraspinal mechanisms of gabapentin, Takasu *et al.*²⁷ recently reported that gabapentin reduced γ -aminobutyric acid-mediated synaptic transmission in locus ceruleus neuron through presynaptic, consistent with gabapentin-induced disinhibition.

Muscarinic Dependency of Gabapentin Analgesia and Combination with Donepezil

Stimulation of spinal cholinergic circuits by activation of spinal α_2 adrenoceptors is widely documented in humans and animals.⁷ Consistent with a previous report in mice with peripheral nerve injury,⁸ oral and intracerebroventricular gabapentin analgesia were completely blocked by intrathecal atropine in the current study. Sensitivity to atropine inhibition of spinal α_2 -adrenoceptor activation differs between normal and neuropathic pain animals. We previously reported that clonidine increased acetylcholine release in spinal cord slices from nerve-injured but not normal rats.²⁸ This agrees with the previous observation that the antihypersensitivity effect of intrathecal clonidine to mechanical stimuli is abolished by intrathecal atropine in nerve-injured rats, but its antinociceptive effect is not inhibited by atropine in normal rats.^{19,20}

Enhanced cholinergic mechanisms of analgesia in the neuropathic pain condition have been previously described. Peripheral nerve injury results in novel cholinergic circuits that underlie α_2 adrenoceptor-mediated analgesia⁷ and increased expression of cholinergic receptors on primary sensory afferents,²⁹ which could produce analgesia when stimulated at their peripheral terminals or those in the spinal cord. Cholinesterase inhibitors produce acute analgesia in humans and animals.⁷ Our previous study showed that chronic oral donepezil administration maintained efficacy over 2 weeks, and this treatment did not lead to desensitization of muscarinic receptor-coupled G proteins in brain or spinal cord,¹¹ suggesting that donepezil does not cause tolerance. Moreover, patients with chronic pain often have cognitive impairment,³⁰ and donepezil improves cognitive function in Alzheimer dementia patients.¹² Based on these encouraging data, we are currently examining the efficacy of oral donepezil to treat pain and improve cognition in patients with chronic pain. Although intravenous physostigmine³¹ and intrathecal neostigmine¹³ produce analgesia in humans, they are not used clinically because of commonly occurring nausea.

Because rats do not show nausea, we could not determine the therapeutic ratio of donepezil in this species. However, the current study tested the range of oral doses in rats which produce cholinesterase inhibition of similar degree to therapeutic doses in patients.^{32,33}

The current study showed strong synergy between oral gabapentin and donepezil in rat after SNL, extending previous observations showing potentiation between gabapentin and neostigmine in mice after partial sciatic nerve ligation⁸ and after acute inflammation induced by plantar injection of formalin.³⁴ Our recent study demonstrated that oral donepezil produces analgesia by actions in the spinal cord but not in supraspinal or peripheral sites.¹¹ Because synergy between gabapentin and donepezil was completely blocked by intrathecal atropine, analgesia by the combination of these two drugs was likely mediated by spinal cholinergic activation. Although rats showed normal behavior such as grooming and exploration activity after oral donepezil and gabapentin administration, the current study provides little guidance on whether this combination would or would not produce side effects in humans. Others have shown that gabapentin (300 mg/day) and donepezil (5–10 mg/day) improve behavioral disorders in Alzheimer patients without side effects,³⁵ which is encouraging.

In summary, gabapentin acts supraspinally after oral administration to activate the descending bulbospinal noradrenergic pathway, and its antihypersensitivity effect is strongly dependent on the activity of spinal cholinergic circuits in rats after SNL. The combination of gabapentin and donepezil produces synergistic analgesia, mediated by spinal muscarinic receptor activation. Given the safety profiles of these drugs in frail patients and the strong synergy observed in this study, we suggest that a clinical trial of this combination is warranted.

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